A Low-Cost, High-Throughput Polyacrylamide Gel Electrophoresis System for Genotyping with Microsatellite DNA Markers

D. Wang, J. Shi, S. R. Carlson, P. B. Cregan, R. W. Ward, and B. W. Diers*

ABSTRACT

Microsatellite DNA markers are widely used in genetic research. Their use, however, can be costly and throughput is sometimes limited. The objective of this paper is to introduce a simple, low-cost, high-throughput system that detects amplification products from microsatellite markers by nondenaturing polyacrylamide gel electrophoresis. This system is capable of separating DNA fragments that differ by as little as two base pairs. The electrophoresis unit holds two vertical 100-sample gels allowing standards and samples from a 96-well plate to be analyzed on a single gel. DNA samples are stained during electrophoresis by ethidium bromide in the running buffer. In addition, one of the gel plates is UV-transparent so that gels can be photographed immediately after electrophoresis without disassembling the gel-plate sandwich. Electrophoresis runs are generally less than two hours. The cost per gel, excluding PCR cost, is currently estimated at about $2.60, or less than $0.03 per data point. This system has been used successfully with soybean [*Glycine max* (L.) Merr.] and wheat (*Triticum aestivum* L.) microsatellite markers and could be a valuable tool for researchers employing markers in other species.

Microsatellite markers, also referred to as simple sequence repeat (SSR) DNA markers, are widely employed in linkage map construction, quantitative trait loci (QTL) mapping, and the analysis of genetic diversity (Wang et al., 2001; Cregan and Quigley, 1997; Temnykh et al., 2000; Roder et al., 1998; Marino et al., 1995). Microsatellites are direct tandem repeated sequences of DNA with a repeat size ranging from one to six base pairs. Microsatellites have been found to be both abundant and widely distributed throughout the genomes of many higher plants and animals (Burow and Blake, 1998). The number of tandem repeats in a microsatellite is highly variable among individuals in a species and is the basis for the length variability of the marker.

The length polymorphism of a microsatellite marker is commonly detected through polymerase chain reaction (PCR) amplification using primers flanking the microsatellite, followed by electrophoresis to determine the length of the PCR product. There are several separation methods currently employed to determine the length of amplification products among which polyacrylamide gels are commonly used. The amplification products in polyacrylamide gels are typically visualized with radioactive labeling, fluorescent dye labeling, and silver staining. However, these visualization methods require either expensive or hazardous radioactive chemicals and are time-consuming. Electrophoresis with MetaPhor agarose gels (Cambrex Corporation, East Rutherford, NJ) has been used to separate alleles of microsatellite markers, but the resolution is lower than nondenaturing polyacrylamide gels and the cost is currently five times more than that of nondenaturing polyacrylamide gels. Capillary electrophoresis also has been used to determine length polymorphisms of microsatellite markers (Marino et al., 1995), but this method requires sophisticated instruments and fluorescently tagged primers, which are expensive. Here we describe an inexpensive and relatively high-throughput system developed for the purpose of genotyping with microsatellite markers.

MATERIALS AND METHODS

The Electrophoresis Unit

The electrophoresis unit was custom-designed and manufactured by C.B.S Scientific Co. (Del Mar, CA) according to our requirements (Fig. 1). C.B.S Scientific Co. now markets this "MEGA-GEL High Throughput Vertical Unit" (model C-DASG-400-50). The unit holds two gels vertically and has a rotating base for easy access to both gels. The two gels can be run either simultaneously or independently. The gels are cast and run between glass plates that are 50 cm wide and 22 cm high. The back plate (round-cornered plate) is UV transparent so that gel photography can be done without disassembling the gel-plate sandwich. Each gel has the capacity for 100 samples so that all samples from a 96-well plate can be loaded in one gel. The sample wells are spaced 4.5 mm apart for loading with a standard 8- or 12-channel pipette.

The Protocol

The nondenaturing polyacrylamide gel is cast in a glass plate sandwich (Fig. 1) with a 1.5-mm-thick spacer on each vertical side, a gel-wrap gasket (Catalogue No. VGE-15XX, CBS Scientific, Del Mar, CA), and six spring clamps. The gel-wrap gasket is mounted on the bottom and side edges of the back plate and sandwiched between the two glass plates to form a leak-proof seal. The gasket replaces the need for tape. The glass plates are cleaned with the household glass cleaner Windex (SC Johnson, Racine, WI) and wiped with disposable low-lint Kimwipes (Kimberly-Clark, Dallas, TX). The plate sandwich is assembled similar to other polyacrylamide gels with spacers and clamps. Two medium office binder clips are placed at the plate sandwich bottom to provide a tight gasket seal and provide additional support during pouring. The plate sandwich stands upright using the binder and spring clamps at the bottom for support.

Approximately 180 mL of gel solution is needed to cast each gel. The final concentration of each gel is 6% (w/v)

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Abbreviations: bp, base pair; ITMI, International Triticeae Initiative; PCR, polymerase chain reaction; QTL, quantitative trait loci; SSR, simple sequence repeat.

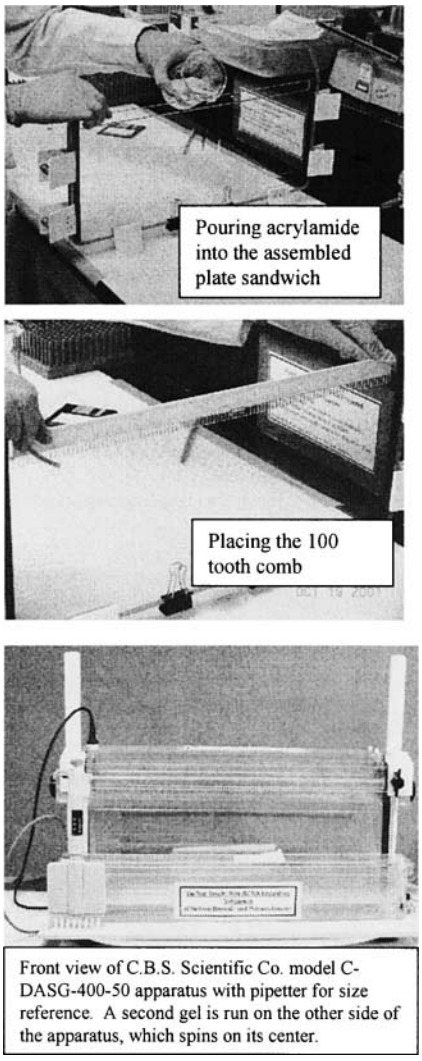

Fig. 1. Images of the gel system (DASG-400-50, C.B.S. Scientific Co).

acrylamide/bis-acrylamide (19:1), 0.5× TBE buffer (Sambrook et al., 1989), 0.07% (w/v) ammonium persulfate, and 0.08% (w/v) TEMED. Immediately after the incorporation of the ammonium persulfate and TEMED, the gel solution is mixed and poured directly between the glass plates. A 100-tooth comb is placed between the plates and two additional spring clamps are used to hold the comb tightly against the back plate. The plate sandwich with gel solution is then kept at room temperature for about 40 min to allow gel polymerization.

After gel polymerization, the clamps, binder clips and gel-wrap gasket are removed from the glass plates. The plate sandwich is then placed in the gel system for electrophoresis. The comb remains in the gel until just before sample loading. Gels are run with about 500 mL of running buffer (0.5× TBE) added to each of the upper and lower reservoirs of the gel system. At this point, the gel can be stored for up to two days before running. Just before electrophoresis, 50 μL of 10 mg mL^{-1} of ethidium bromide is added to the lower reservoir. Since ethidium bromide migrates in the opposite direction of DNA, it will move through the gel during electrophoresis.

DNA samples are loaded with a standard 8- or 12-channel pipette. Type IV gel-loading buffer described by Sambrook et al. (1989) is added to the DNA samples before loading. Loading volume can be up to 30 μL. Electrophoresis is performed at approximately 350 V for 1 to 2 h. A small household fan (12 inch) is used to cool the system by circulating air between the plate sandwiches. When the electrophoresis is completed, the plate sandwich is taken off the gel system and visualized on a UV light box with the UV transparent back plate on the bottom. Gel photography takes place with the gel remaining in the plate sandwich. The DNA bands are visualized with 254-nm UV light. A Canon PowerShot G1 digital camera (Canon U.S.A., Inc., Lake Success, NY) with a UV filter and a deep-yellow-ethidium-bromide filter was used for gel photography. The camera is operated through a desktop computer with software provided by the camera manufacturer. Because of the size limit of our UV light box, two photographs are taken to capture the entire 100-lane gel.

Table 1. Lengths in base pairs (bp) of alleles of six microsatellite markers for W7984 and Opata, the parents of ITMI population.

	Allele size bp	
Marker	**W7984**	**Opata**
Xbarc218	**210**	**212**
Xbarc222	**182**	**185**
Xbarc219	**208**	**220**
Xbarc196	**145**	**163**
Xbarc013	**142**	**140**

For comparison purposes, the commonly used denaturing gel with silver staining was also evaluated (Hazen et al., 2002). The PCR products were resolved in a 6% (w/v) denaturing polyacrylamide gel with a BioRad (Hercules, CA) sequencing gel system. The gel was run in 1× TBE buffer at 85 W for 2 h and then silver stained according to the protocol provided by Promega (Madison, WI).

Microsatellite Markers and PCR Reaction

Wheat microsatellite markers developed by Drs. Q.J. Song and P.B. Cregan at the Beltsville Agriculture Research Center of USDA-ARS were tested on RILs of the International Triticeae Mapping Initiative (ITMI) population derived from a cross between the varieties Opata 85 and W7984 (Nelson et al., 1995). This population has been employed by others for linkage map construction in wheat (Nelson et al., 1995; Van Deynze et al., 1995). Table 1 shows the microsatellite markers and the allele sizes of the two parents.

The PCR amplification was performed in a PTC-0220 DNA Engine Dyad Peltier Thermal Cycler manufactured by MJ Research (Waltham, MA). The reaction volume was 20 μL and reaction mixture contained 45 ng of template DNA, 2 μ*M* primer, 30 m*M* MgCl, 3 m*M* each of dNTP, 2.5 units of Taq polymerase, and 1× PCR buffer that was provided by the Taq polymerase manufacturer. The PCR was run with an initial denaturation at 95°C for 2 min, followed by 38 cycles of 25 s denaturation at 94°C, 25 s of annealing at annealing temperature specified for each primer pair, and 45 s extension at 70°C. The final cycle was followed by a 10-min extension at 72°C. The PCR product was held at 4°C before analysis.

RESULTS AND DISCUSSION

The MEGA-GEL High Throughput Vertical Unit system was able to separate alleles of microsatellite markers with a size difference of three base pairs (bp) clearly. Fig. 2 shows the separation of two alleles of the microsatellite marker Xbarc196 after electrophoresis at 350 V for 1.5 h. The two alleles of Xbarc196 differed by 18 bp (145 and 163 bp). Fig. 3 shows the separation of 182 and 185 bp alleles of Xbarc222 after electrophoresis at 350 V for 1.5 h.

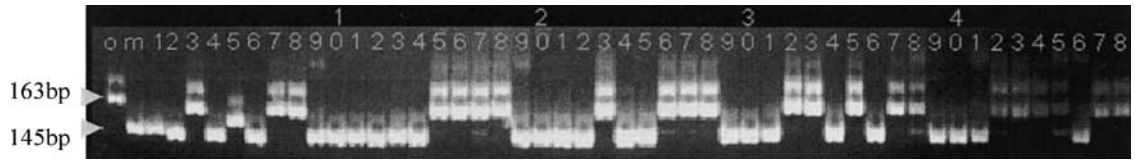

Fig. 2. Gel image for microsatellite marker Xbarc196 with allele sizes of 145 and 163 bp. The image was taken after 1.5 h of electrophoresis at 350 V. The DNA was stained during electrophoresis with the running buffer containing 0.1 μg mL^{-1} of ethidium bromide. The DNA bands were visualized with 254 nm UV light. Lane o: Opata; lane m: W7984; lanes 1-48: progeny of the ITMI population.

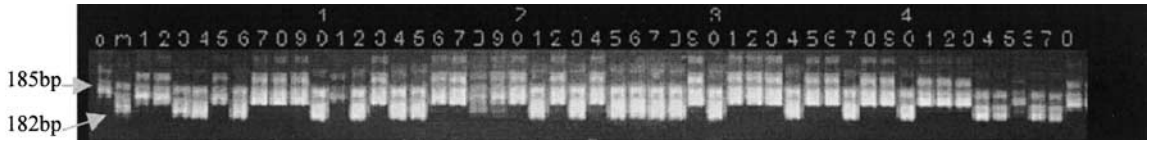

Fig. 3. Gel image for microsatellite marker Xbarc222 with allele sizes of 182 and 185 bp. The image was taken after 1.5 h of electrophoresis at 350 V. DNA staining, visualization, and lane references are the same as described in the caption for Fig. 2.

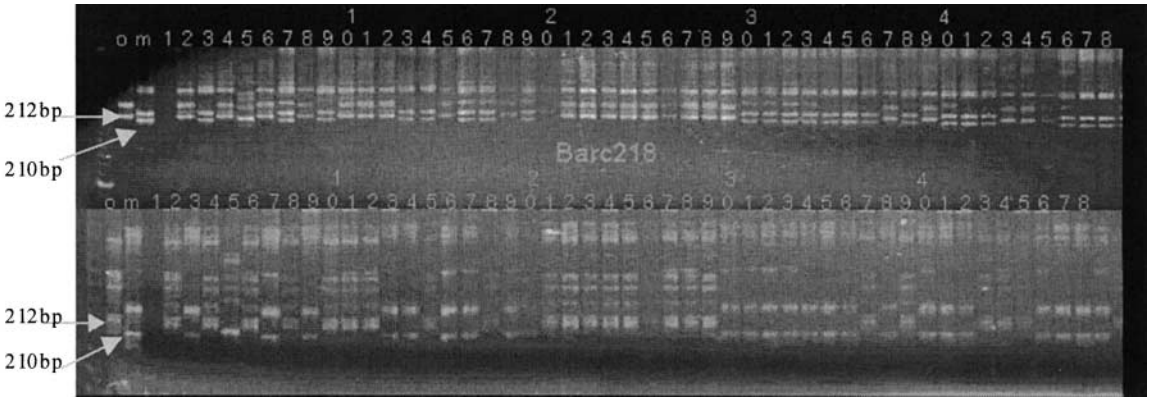

Fig. 4. Gel image for microsatellite marker Xbarc218 with allele sizes of 210 and 212 bp. The top section of the image was taken after 1 h of electrophoresis at 350 V and the bottom section of the image was taken after 3 h of electrophoresis at 350 V. DNA staining, visualization, and lane references are the same as described in the caption for Fig. 2.

When two alleles differed by only 2 bp, longer electrophoresis times were needed for clear separation of the alleles. Fig. 4 shows the difference of one versus three-hour electrophoresis at 350 V in the separation of 210 and 212 bp alleles of marker Xbarc218. One hour of electrophoresis barely separated the two alleles (Fig. 4, top) while 3 h of electrophoresis clearly separated the two alleles (Fig. 4, bottom).

To increase throughput further, two or three amplification products can be loaded simultaneously in the same well if the allele sizes of these markers are known to not overlap. Alternatively, if the allele sizes of the markers are similar, one sample set can be electrophoresed for a period of time, and then additional samples loaded into the same wells followed by additional electrophoresis. Fig. 5 shows the results obtained by successive microsatellite sample loading using marker Xbarc196. The second sample set was loaded one-hour after the initial set and was followed by an additional 1.5 h of electrophoresis at 350 V. The two alleles were clearly separated for both sample sets. Better separation of the alleles in the first loading are due to their longer electrophoresis time (Fig. 5).

In addition to the desired microsatellite amplification fragments, higher molecular weight minor bands (faint or heteroduplex) are also observed in Fig. 2 through 5. Faint bands are believed to be PCR artifacts and are observed in homozygous individuals while heteroduplex bands only occur in heterozygotes (Rodriguez et al., 2001). Heteroduplex bands are caused by the reannealing of noncomplementary strands during later rounds of PCR (Rodriguez et al., 2001). The banding pattern of these minor bands is generally consistent and can be useful during gel scoring for genotype verification.

The nondenaturing electrophoresis system has nearly the same resolution as that obtained with denaturing acrylamide gels and silver staining. Fig. 6 shows the results of using both systems to separate the 140- and 142-bp alleles of marker Xbarc013. The upper section of Fig. 6 was the gel image captured after UV exposure of the nondenaturing electrophoresis system. The bottom section of Fig. 6 was the image developed by silver staining after electrophoresis of a denaturing sequencing gel. Although the nondenaturing system tends to produce slightly more diffuse bands than the denaturing system, there is little difference in resolution (Fig. 6).

The results clearly demonstrate that the system has a resolution to distinguish alleles of most microsatellite markers. This system is ideal for laboratories currently using denaturing polyacrylamide gel system and silver staining or fluorescent dye labeling for genotyping with microsatellite markers.

The cost of using this system for genotyping is low. The gel ingredients cost approximately $2.60, and a gel can be used to obtain 100 data points without multiplexing. This brings the gel cost per data point to less

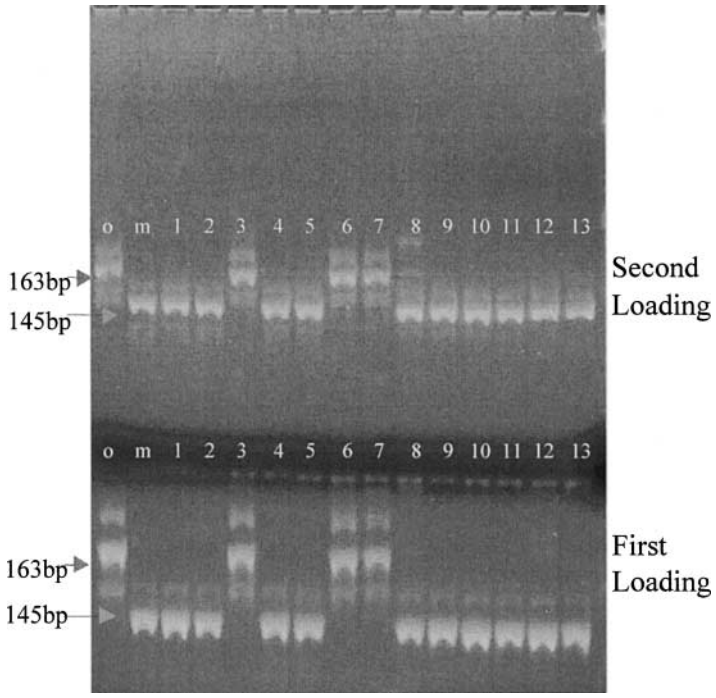

Fig. 5. Gel image with two successive loadings of the same microsatellite sample set using marker Xbarc196. The second sample set was loaded 1 h after the initial samples and electrophoresis was continued for an additional 1.5 h at 350 V. DNA staining and visualization are the same as described in the caption for Fig. 2. Lane o: Opata; lane m: W7984; lanes 1-13: progeny of the ITMI population.

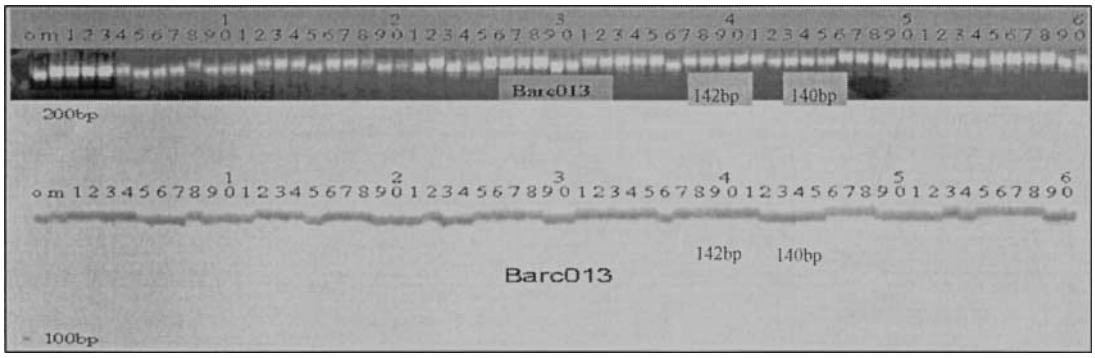

Fig. 6. Comparison of gel images from our nondenaturing system (top) and a denaturing silver staining system (bottom). Electrophoresis, DNA staining, and visualization for the nondenaturing system were the same as described for Fig. 2. For the silver-staining system, the electrophoresis was performed in a 6% (w/v) denaturing polyacrylamide gel with the BioRad sequencing gel system. The gel was run in 1× TBE buffer at 85 W for 2 h and then silver stained according to the protocol provided by Promega (Madison, WI). Lane o: Opata; lane m: W7984; lanes 1-60: progeny of the ITMI population.

than $0.03. The gel system itself is also inexpensive with a current price of less than $2000.

The throughput of this system for genotyping is relatively high. With five of these systems in our laboratory, a person can routinely run 10 gels a day with hands-on time of less than 5 h per day. Therefore, without multiplexing, a person can obtain 1000 data points in 5 h.

This system compares very favorably with denaturing gels and silver staining techniques that are widely used in many laboratories for genotyping with microsatellite markers. For the silver staining system, a person can normally handle only one gel per day and only 49 samples can be loaded on the gel. The gel ingredients for a denaturing sequencing gel cost about $3.00 while the silver staining costs are about $7.00. Moreover, the silver staining process takes approximately two hours.

An additional benefit of the nondenaturing system is the ease of use and the ability to use standard laboratory equipment. Denaturing gels require PCR products to be denatured before loading. In addition, denaturing gels must be run at constant temperatures. A specialized power supply or constant vigilance is needed to maintain this temperature. Nondenaturing gels, in contrast, do not require samples to be denatured and can be run on a standard power supply.

This system has been used successfully with both soybean and wheat microsatellite markers (Wang et al., 2001) and could be a valuable tool for other researchers employing microsatellite markers.

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